Data from Short-Term Tests on Motor Vehicle Exhausts

by Ulf Rannug*

The mutagenicity of motor vehicle exhausts has been studied by using *Salmonella typhimurium* strains TA 98 and TA 100. Acetone extracts of the particulate phase and the gas phase have been tested in the presence and absence of a metabolizing system (S9).

The particulate phases from medium- and heavy-duty diesel vehicles were tested. The vehicles were driven according to a modified 13 mode test, and the particulate phase was sampled at mode 6 (maximum load and intermediate engine speed) and mode 12 (10% load and rated speed). In mode 6 all vehicles gave approximately the same mutagenicity in strain TA 98 (50,000–90,000 revertants/kW-hr) as well as in TA 100 (200,000–360,000 revertants/kW-hr). A higher mutagenic effect, in some cases up to 10 times, was seen with mode 12.

Light-duty vehicles of different year models were tested using different fuel/engine combinations. The vehicles were driven according to FTP 72 or ECE driving cycle. Cold starts at two different temperature levels, approx. 0 °C and 23 °C, respectively, were also compared. Based on the mutagenicity of the particulate extracts (given as revertants per km), the light-duty vehicles could be divided into three main groups. The first group, the high mutagenicity group, giving 100,000–700,000 revertants/km, consists only of diesel cars. In the medium mutagenicity group, giving between 20,000 and 100,000 revertants/km, different gasoline fuels are placed, i.e., leaded and lead-free gasoline as well as alcohol/gasoline fuels. Two other fuels, methanol (M95) and propane (LPG), constitute the low mutagenicity group, giving less than 20,000 revertants/km. Fuels from the medium effect group will produce a particulate phase with low mutagenicity if the vehicle is equipped with a three way catalyst with closed loop, or fuel injection.

The cold start temperature did not change this classification, since all samples gave a somewhat higher mutagenic effect at the low temperature.

With the ECE driving cycle, much lower mutagenicity was noted with the diesel cars than in the tests with the FTP-72 driving cycle, at least with tester strain TA 98. On strain TA 100 the diesel exhaust samples still showed a much higher mutagenicity than other samples.

Acetone extracts of the gas phase from diesel and gasoline exhaust (trapped in ice/water condensers and CO_2/ethanol condensers) also gave mutagenic effects. The contribution of the gas phase to the mutagenic effects seems to be more important in the absence of S9 and more important in the case of gasoline exhausts.

Introduction

The Swedish Government Committee on Automotive Air Pollution and the Swedish National Environment Protection Board have initiated research on possible health effects of motor vehicle emissions and air pollution. Although several toxic effects are considered, genotoxic effects constitute one of the important parts. Normally, when only one chemical is involved, the way from the initial

risk identification to the final risk assessment is difficult. When it then comes to a more complex exposure situation, as in the case of automobile emission and air pollution, this process is even more complicated. Thus, the first step, the risk identification, may also include difficulties not normally encountered at this level. A screening of the genotoxic effects of automobile emission must be carried out with a relatively sensitive and simple test system. This report gives a brief summary of some results attained at this first level, i.e., screening and characterization of different automobile emissions. Special emphasis has been put upon the

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present situation in the Scandinavian countries. Most work has hence been carried out on today's cars, but the future situation has been taken into consideration in terms of different fuel-engine combinations. Some aspects on sampling methods for *in vitro* testing of exhaust emission will also be given.

Material and Methods

Vehicles and Driving Cycles

The vehicles used have kindly been put to the disposal of Swedish National Environment Protection Board, Motor Vehicle Section, Studsvik by Volvo Car Corp., Saab-Scania AB and Swedish Motor Fuel Technology Co., Philipsons and Esso. All the chassis dynamometer tests were carried out at the Motor Vehicle Section of Studsvik. The samples were taken when the light-duty vehicles were driven according to the U.S. driving cycle, average speed 31.7 km/hr (FTP 1972), with cold (20–30°C) starts if nothing else is stated. Sweden has adopted the US Test Procedure (FTP 1973-74) for certification testing of motor vehicles of 1976 and later year models. For comparison test series with cold starts at a lower (-11 to +7°C) ambient temperature were carried out. Another driving cycle, the ECE-driving cycle (average speed 18.7 km/hr) adopted by the ECE (United Nations Economic Commission for Europe), was also used for comparison. Before a test series the vehicles were conditioned according to a specified procedure (road driving at approx. 90 km/hr for 45 min). The medium- and heavy-duty diesel vehicles were operated on a chassis dynamometer at a modified 13 mode test which involves 13 steady-state operations with different loads and speeds of the engine. Samples for mutagenicity testing were taken at mode 6 (maximum load and intermediate speed) and mode 12 (about 10% load and rated speed). Further details of the vehicles and fuels are given elsewhere (*1*).

Sampling Methods

The exhaust samples were taken after dilution by means of a dilution tunnel, constructed at the Motor Vehicle Section at Studsvik according to the specifications in Federal Register (*2*). The approximate diluting ratio was 1:10 for the gasoline vehicles and about 1:7 for the light-duty diesel vehicles. Each run was performed three times. Different tunnels were used for light-duty diesel vehicles, medium- and heavy-duty vehicles and other vehicles, respectively.

Particulate Phase. The particulate matter was trapped on glass fiber filters (Gelman A/E). These were washed in ethanol and heated for 1 hr at 120°C

before being used. After sampling the filters were either extracted within 2 days or stored at -20°C and extracted within 3 weeks. Each filter was extracted with 250 mL acetone in a Soxhlet apparatus for 16 hr. Siphoning time was 15 min. The volume was then reduced at 37°C under a stream of nitrogen and adjusted to correspond to a given volume of raw exhaust, normally 1 mL of extract was equivalent to either 50 L of exhaust (diesel) or 100 or 150 L of exhaust (gasoline). When PAH-analysis (see below) was carried out parallel filters from the same run were extracted and fractionated according to the procedure given elsewhere in this volume (*3*).

Gas Phase. Parallel to the particulate sampling gas phase trapping was also carried out in some experiments. The cryogradient technique and procedure used in these experiments have been described in detail elsewhere by Stenberg et al. (*3*). Briefly, the following equipment is used. The sampling probe connected to the dilution tunnel permits particulate sampling on three identical filters. Usually one or two of the filter holders were used for sampling of particulates for mutagenicity testing. For the gas-phase enrichment, a sampling system with two consecutive condensers (an ice/water condenser, and a CO_2/ethanol condenser, respectively) was used. Each condenser has a cooling area of approximately 0.5 m^2. The maximum sampling velocity with this system is 100 L gas/min. After each run the condensers were extracted with acetone in a special Soxhlet apparatus (*3*). The volume of the extracts was reduced and adjusted as described for the particulate extract.

Test Procedure

The *Salmonella typhimurium* strains TA 98 and TA 100 used in this investigation were kindly provided by Dr. B. N. Ames, University of California, Berkeley. Basically the plate incorporation-assay according to Ames et al. (*4*) was followed with minor modifications. Thus histidine (0.1 μmole/plate) and biotin (0.1 μmole/plate) were added to the minimal medium instead of to the soft agar. Series for determining the toxic effects were also included. In these series the overnight culture was diluted 2 × 10^6 times and the soft agar mixture was poured onto nutrient agar (antibiotic medium 3, Difco) plates instead of minimal agar plates. Liver preparations (S9-fractions) from Aroclor pretreated male rats of strain Sprague-Dawley were used as metabolizing systems. The extracts were tested at three different concentrations normally corresponding to 2.5, 5.0 and 7.5 L of exhaust in the case of gasoline or 1.25, 2.5 and 5.0 L in the case of diesel. All plates were incubated at 37°C for 48 hr before scoring.

The results were analyzed by regression analysis from the linear portion of the curves and the results shown in tables and figures (number of revertants per liter exhaust, per km or per kW-hr) are calculated from the slope of the regression line.

Results

Particulate Phase

In Figure 1 the result of the mutagenicity tests of particulate extracts from different light-duty vehicles and fuels is shown. Four different gasoline powered cars were tested. The cars were of different year model and fueled with either leaded or lead-free gasoline. One car was equipped with a three-way catalyst and closed loop system for the fuel injection control. In Figure 1 the mutagenic effect is given in revertants per liter exhaust. The corresponding results in revertants per kilometer driven is shown in Figure 2.
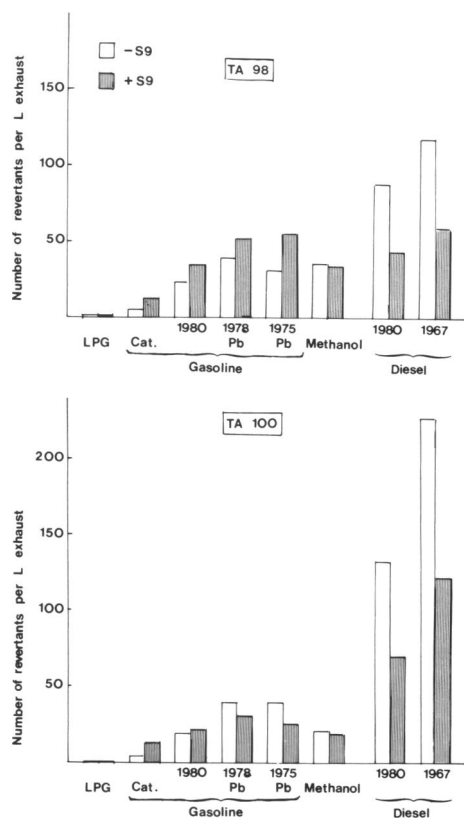

FIGURE 1. Mutagenic effects of particulate extracts (acetone) of exhaust samples from different light-duty vehicles on *Salmonella typhimurium* TA 98 and TA 100 in the presence and absence of a metabolizing system (S9). The exhaust was sampled after dilution and the vehicles were operated on a chassis dynamometer, using the FTP-72 driving cycle with cold starts at about 0°C.
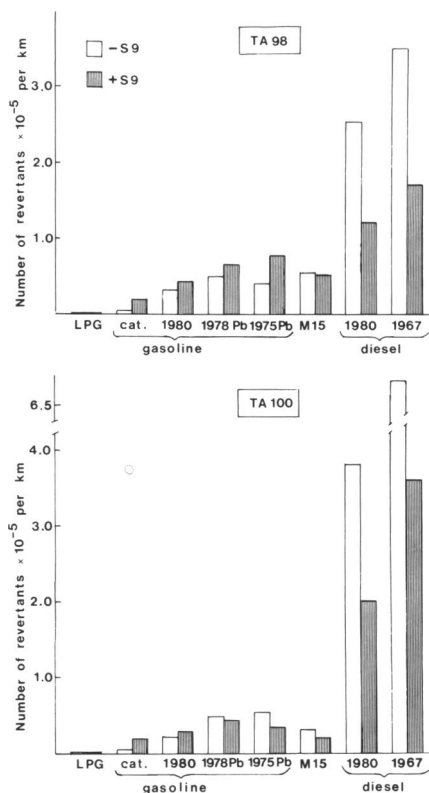

FIGURE 2. Mutagenic effects of particulate extracts of exhaust samples as in Fig. 1, but given as number of revertants/km driven.

Apart from the catalyst equipped car, which gave a lower mutagenic effect, the mutagenicity of the different gasoline fueled cars was quite similar. Also when a lead-free gasoline with 15% methanol was used, the particulate extract of the exhaust gave approximately the same mutagenic effect. For comparison, the mutagenic effect of an extract from a car fueled with liquified petroleum gas (LPG), about 95% propane, is shown. This car gave 2 revertants/L or less than 3000 revertants/km on both strains. The two light-duty diesel vehicles, on the other hand, gave the highest mutagenic response on both strains. Thus, the different cars tested can roughly be put into three categories based on the mutagenicity of their particulate extracts. The cars giving the lowest effects were the LPG car and the catalyst car. These cars showed less than 20,000 revertants/km. For the light-duty diesels more than 100,000 revertants/km were found in all cases. The highest effect, between 380,000 and 680,000 revertants/km, was seen in strain TA 100 in the absence of S9 (Fig. 2). These cars thus constitute the high effect group (100,000-700,000 revertants/km) and the gasoline cars the medium effect group. With

this relatively limited number of samples, no major differences could be seen between cars fueled with leaded or lead-free gasoline. Likewise, a distinction between different year models among the gasoline cars based on mutagenic effects was not possible, although the newest car tested also gave the lowest effect in both strains. Also the newer diesel, (1980 year model) gave lower mutagenic effects on both strains than the older diesel, 1967 year model. A comparison of the mutagenic effects with and without S9 for the different cars gave the following results. A 50% reduction in mutagenicity was noted with the diesel extracts when the S9-fraction was added. For the gasoline-fueled cars the mutagenicity with and without S9 was similar, although a small increase with S9 was seen in most cases at least with strain TA 98.

To test the effect of ambient temperature on the mutagenicity of the exhaust from light-duty vehicles a set of experiments with cold starts at the two temperature levels about 0°C and about 23°C, respectively, were carried out using the FTP-72 driving cycle. The results are shown in Figure 3. Both with
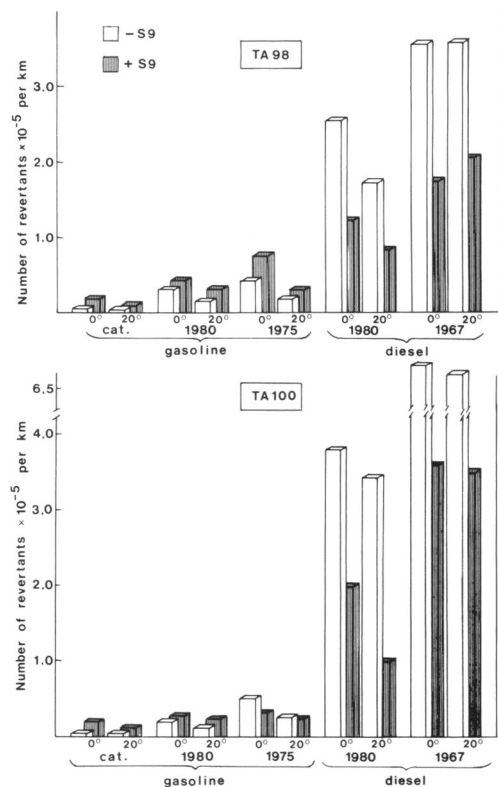
TA 98 and TA 100, the extract from the old diesel car (1967) showed similar mutagenicity at the two temperatures. All other cars—including the newer diesel car—produced a more mutagenic (between 1.2 and 2.3 times) particulate extract at the low temperature. This was seen with both the tester strains TA 98 and TA 100.

Tests were also carried out to investigate the influence of the driving cycle on the mutagenicity of the particulate extract. In Figure 4, the mutagenic effects of particulate extracts from gasoline- and diesel-powered light-duty vehicles driven according to FTP-72 and the ECE driving cycle, respectively, are shown. In all cases, except with the 1975 gasoline car, cold starts at about 0°C were used. For the 1975 car in Figure 4 cold starts at about 23°C were employed. The two light-duty diesel vehicles both gave lower mutagenic effects with the ECE driving cycle. This was seen on both tester strains.

FIGURE 3. Comparison of the mutagenic effects of particulate extract samples taken at about 0 °C and about 23 ° C from gasoline and diesel car exhaust on *Salmonella typhimurium* TA 98 and TA 100 in the presence and absence of a metabolizing system (S9). The driving cycle used was FTP-72.
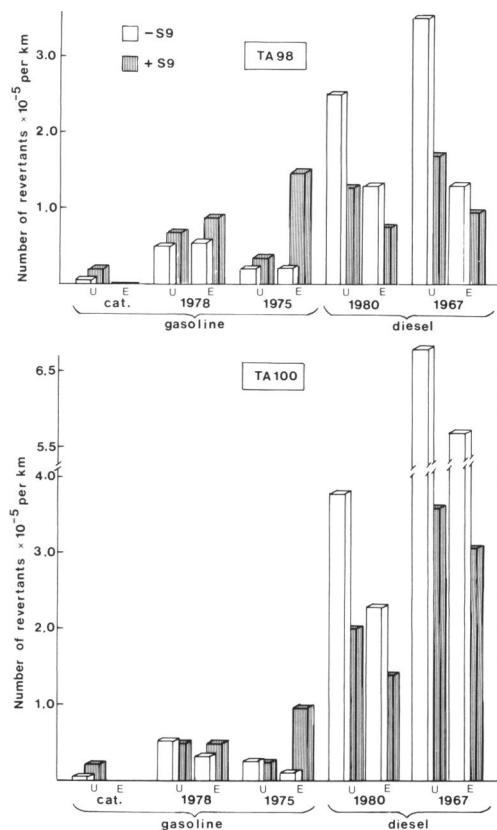

FIGURE 4. Comparison of the mutagenic effect of particulate extracts from the emission of light-duty vehicles driven according to driving cycle FTP-72 (U) and ECE (E) on *Salmonella typhimurium* TA 98 and TA 100. The mutagenicity was tested both in the presence and in the absence of a metabolizing system (S9). All samples were taken at 0°C except for those from the 1975 gasoline car all of which were taken at 20 ° C.

The reduction in strain TA 98 was between 50% and 60% both with and without S9, while the reduction was smaller in strain TA 100. The catalyst-equipped car produced a particulate extract with much lower mutagenicity (revertants per liter exhaust or per km) when the ECE driving cycle was used. Thus, strain TA 100 showed no mutagenic response at all with this driving cycle (Fig. 4). With the other gasoline cars no consistent difference was noted between the two driving cycles if both tester strains are considered. At the comparison with starting temperature at 23°C instead of 0°C (year model 1975, Fig. 4) the mutagenic effect with S9 was higher with the ECE driving cycle.

Mutagenicity tests have also been performed with heavy and medium duty diesel vehicles. Two trucks, one new and one of older year model, of each category were tested. The vehicles were driven

according to the modified 13 mode test and the particulate phase was sampled at mode 6 and 12 (see Material and Methods). The results of these tests are shown in Figure 5. In mode 6 (maximum load and intermediate engine speed) all vehicles gave approximately the same mutagenicity in TA 98 (50,000-90,000 revertants/kW-hr) as well as in TA 100 (200,000-360,000 revertants/kW-hr). For comparison, the same results are also given as revertants per liter exhaust in Figure 6. The corresponding numbers per liter exhaust were thus 22-32 in TA 98 and 80-170 in TA 100, for mode 6.

When the mutagenic effect is given as revertants per liter exhaust there is only a small difference between the two medium duty vehicles in both strains. There is also only a minor difference between mode 6 and 12. The heavy-duty vehicles, however, showed a higher mutagenic effect at mode 6 (between twice and six times higher) than at mode 12 (Fig. 6).

If the comparison is based on revertants/kW-hr, which is more appropriate for this type of vehicle, a higher mutagenic effect, in some cases up to 10 times, is seen with mode 12, i.e., at 10% load and
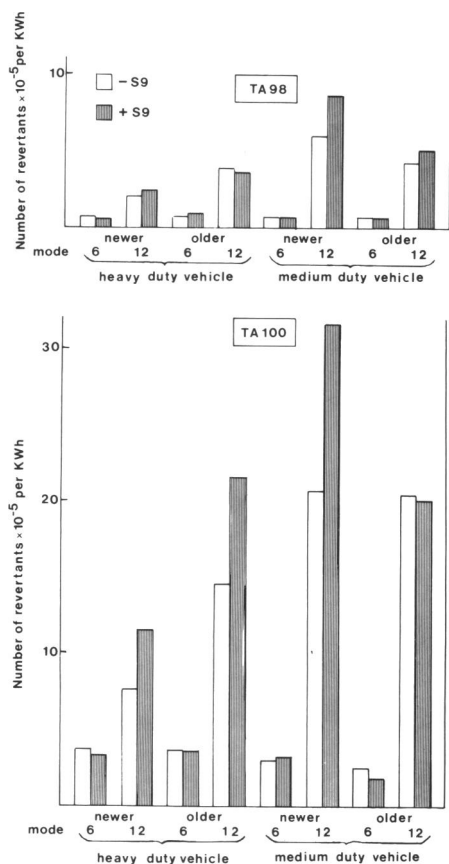

FIGURE 5. Mutagenic effects (given as number of revertants/kWh) of particulate (acetone) extract samples of exhaust from medium- and heavy-duty diesel vehicles on *Salmonella typhimurium* TA 98 and TA 100. The mutagenicity tests were performed both with and without the addition of a metabolizing system (S9). The exhaust samples were taken at mode 6 and 12 of the 13 mode test.
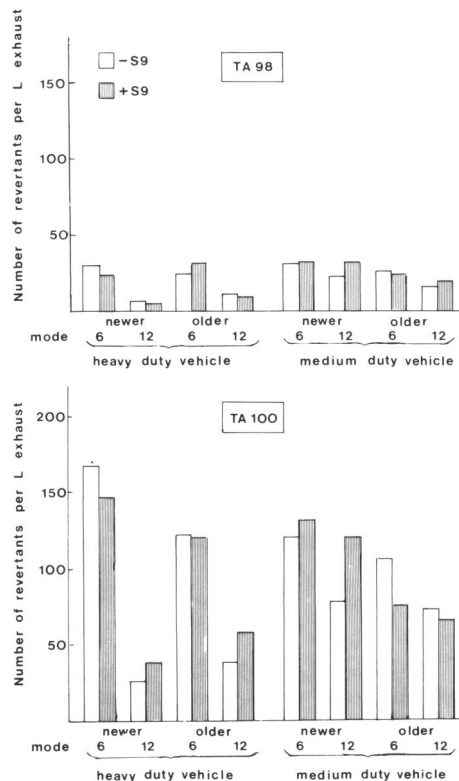

FIGURE 6. Mutagenic effects of particulate extracts of exhaust samples as in Fig. 5, but given as number of revertants/L exhaust.

rated speed. With this mode samples from the old heavy-duty vehicle showed a higher mutagenic effect than the newer vehicle, while the reverse was true for the medium-duty vehicles (Fig. 5).

In Figure 7 the effect of different fuel/engine combinations on the mutagenicity of the particulate extract from light-duty vehicles can be seen. Vehicles and fuels are described in the legend to Figure 7. All vehicles were driven according to the FTP-72 driving cycle with cold starts at about 23°C. The same classification (in a high, medium, and low effect group) based on the mutagenicity of the particulate extract, as mentioned above, can be used also for these samples. Again, the diesel vehicle gave the highest mutagenic effect on both strains, 400,000 rev/km on TA 98 and 500,000 rev/km on TA 100. The medium effect group (Fig. 7) gave less than 100,000 rev/km and consisted of different types of gasoline (G1 and G2). Also the gasoline alcohol fuels, i.e., 23% ethanol or 15% methanol in gasoline, belong to this group. For some of the samples,
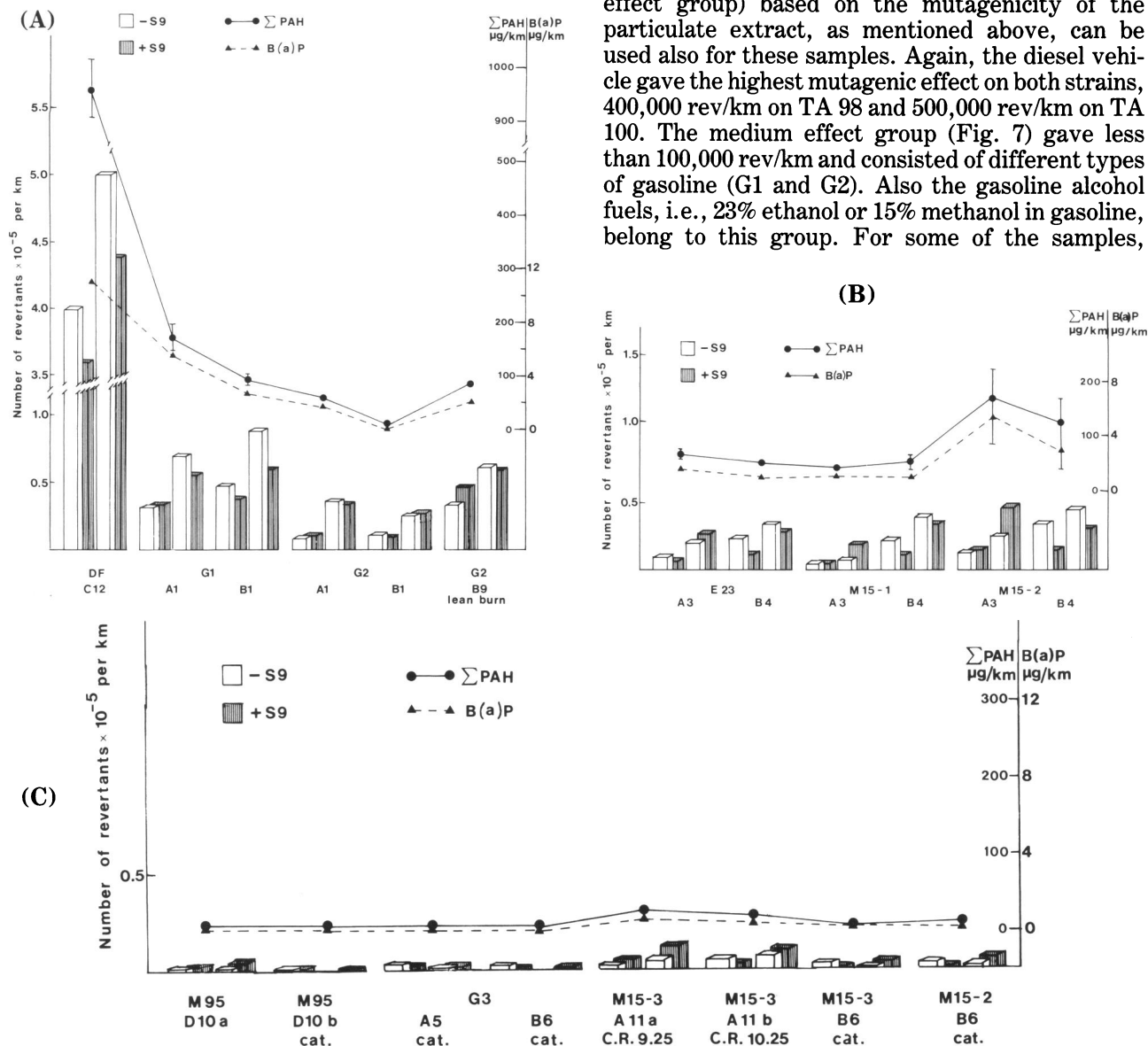

FIGURE 7.   PAH content, B(a)P content and mutagenic effects on *Salmonella typhimurium* of particulate extracts of exhaust emissions from cars operated on different fuels. The FTP-72 driving cycle, cold start at about 23°C was used. The samples were tested both with and without the addition of a metabolizing system on tester strains TA 98 (the two left bars for each type of car) and TA 100 (the two right bars). Types of cars: (Al, B2) carburetor, designed to meet the 1976 Swedish regulations; (A3, B4) the same vehicle as A1 and B2, adjusted for M15/E23; (A5, B6) fuel injection, three-way catalyst, closed-loop; (B9) lean burn engine, fuel injection; (D10a) methanol engine, carburetor; (D10b) the same vehicle as D10a, three-way catalyst, no closed loop; (A11a) fuel injection, without catalyst, closed loop, C-R 9.25; (A11b) fuel injection, without catalyst, closed loop, C-R 10.25; (C12) diesel engine. Types of fuels: (G1) gasoline, leaded; (G2) gasoline, leaded; (G3) gasoline, lead-free; (M15-1) methanol-gasoline fuel, leaded; (M15-2) methanol-gasoline fuel, lead-free; (M15-3) methanol-gasoline fuel, lead-free; (E23) ethanol-gasoline fuel. Further details of the fuel/engine combinations are given elsewhere (1).

however, less than 20,000 revertants/km were found with strain TA 98 (fuel G2, car A1 and B2; fuel E23 car A3; M15-1, car A3 in Figure 7).

All catalyst cars belong to the low effect group (Fig. 7c), i.e., giving less than 20,000 revertants/km. It can be noted that a car without a catalyst and fueled with methanol (M95) also belongs to this group. Likewise, M15 fueled vehicles with fuel injection and closed loop but without catalyst (vehicle A11a and A11b in Fig. 7) gave mutagenic effects characteristic for this low effect group. For comparison, the amount of benzo(a)pyrene [B(a)P, μg/km] and the total amount of PAH are shown. The amount of PAH and the mutagenicity seems to be correlated and the amount of B(a)P for these cases could be used as an indicator of the amount of PAH at least for the gasoline fueled cars. In the diesel exhausts in these experiment the amount of B(a)P was lower relatively to the amount of PAH than for the other exhaust samples.

Gas Phase

So far only the mutagenicity of the particulate phase has been discussed. However, some preliminary experiments have been carried out also with the gas phase from gasoline and diesel fueled light duty vehicles. A more detailed characterization of the gas phase is given elsewhere (5,6). The results given in Figure 8 clearly show that the gas phase contributes to the mutagenicity of the exhaust. This seems to be of more importance in the case of gasoline fueled cars than for diesel cars. The contribution to the direct effect on TA 98 in these experiment was approximately 60%, while for TA 100 the effect of the gas phase varied from 60% up to around 90% of the total effect. In the presence of S9 the effect of the gas phase was somewhat lower. With TA 98, 25-30% of the effect originated from the gas phase. For TA 100, one sample contributed with 20%, while the other two samples contributed with 50-65% of the total mutagenicity. The samples from the light-duty diesel vehicles showed that without S9 25-30% of the mutagenicity originated from the gas phase. In the presence of S9 between 5 and 20% of the mutagenicity came from the gas phase.

Discussion

The results presented in this paper show, that the particulate extracts of exhaust emission from light-duty vehicles fueled with different fuel-engine combinations all give mutagenic effects in either or both of the *Salmonella typhimurium* tester strains TA 98 and 100. The different types of samples
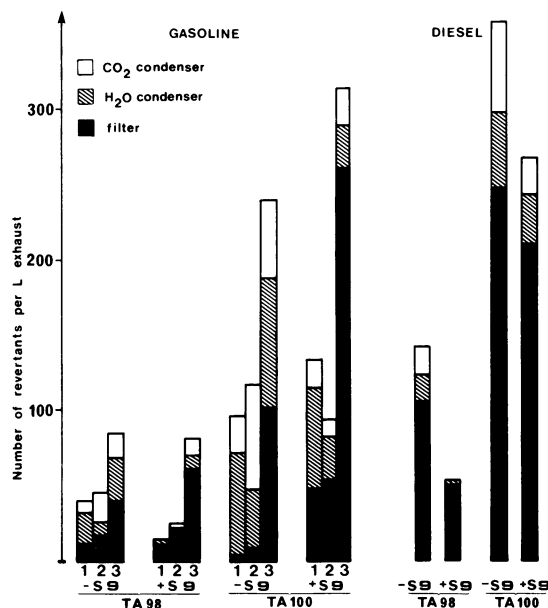

FIGURE 8. Mutagenic effects (revertants per liter exhaust) on *Salmonella typhimurium* TA 98 and TA 100 of the particulate phase (acetone extract) and the gas phase (acetone extracts from H_2O and CO_2 condensers, respectively) of exhaust emission from gasoline and diesel cars. FTP-72 driving cycle (cold start at about 0°C). Three individual tests were carried out with the gasoline car, and one test with the diesel car. All samples were tested with and without the addition of S9. The mutagenicity as well as CO and PAH emissions from the third sample of the gasoline car were much higher than normal and indicate malfunction of the engine.

can be classified into three main groups according to mutagenic potency of the particulate extract. With this classification light-duty diesel vehicles constitute the high mutagenicity group giving between 50-250 revertants/L exhaust or 100,000-700,000 revertants/km. In the second group different gasoline fuels are placed, i.e., leaded and lead-free gasoline as well as gasoline–alcohol fuels giving 10-50 revertants/L or 10,000–20,000 revertants/km. Two other fuels, methanol and LPG, constitute the third group giving less than 10 revertants/L or less than 20,000 revertants/km. When gasoline fueled cars are equipped with a three-way catalyst the mutagenicity of the particulate extracts is reduced to the low level characteristic for the third group. It should be pointed out, however, that this classification is based on the mutagenicity of acetone extract of the particulate phase generated during FTP-72 driving cycles.

Since many factors affect the emission some tests were carried out with another driving cycle as well as at another cold start temperature with the FTP-7 2 driving cycle. It is known that the ambient temperature affects the exhaust emissions and generally

the emission of hydrocarbons and carbon monoxide increases as the ambient temperature decreases (7). The mutagenic effects of the particulate extracts are also somewhat higher at the lower (0°C) temperature. This seems to be a general difference and does not change the classification of the samples in the three mutagenicity groups.

When the light-duty vehicles were driven according to the ECE driving cycle instead of the FTP-72 driving cycle, no uniform increase or decrease in mutagenicity could be seen with the vehicles tested. The diesel cars showed much lower mutagenicity both per liter exhaust and per kilometer driven with the ECE driving cycle. Also the catalyst equipped gasoline car showed lower mutagenicity with this driving cycles compared to the FTP-72 driving cycle (Fig. 4). The other gasoline cars showed similar, or in one case, higher mutagenicity with the ECE driving cycle. One consequence of these driving cycle dependent changes in mutagenicity of the particulate extracts is that with the ECE driving cycle and strain TA 98 no major difference in mutagenicity can be seen between the gasoline exhaust samples and the diesel exhaust samples. On strain TA 100 the diesel exhaust samples still show a much higher mutagenncity than the other samples and can therefore, also by using the ECE driving cycle, be put in the high-effect group, i.e., samples giving more than 100,000 revertants/km. The catalyst-equipped car gave the lowest mutagenic effect with both driving cycles and can therefore in both cases be classified as a low-effect sample.

Although the results show that some factors like ambient temperature and driving pattern affect the mutagenicity of particulate extracts from light duty exhaust emission on the *Salmonella typhimurium* tester strains a rough ranking order for different fuel engine combinations can be made (Fig. 7). The highest mutagenicity is seen with diesel engine and fuel. A medium effect is seen with different types of gasoline or gasoline alcohol fuels (23% ethanol and 15% methanol). The fuels giving the lowest mutagenic effects are LPG and methanol. Also fuels from the medium effect group will produce a particulate phase with low mutagenicity if the vehicle is equipped with a three way catalyst with closed loop (i.e., a oxygen sensor), or fuel injection (Fig. 7c).

One important question in this connection is if this semiquantitative ranking of light duty vehicles still holds if the gas phase is taken into consideration. Our limited data indicate that, under normal conditions, there is still a significant difference between gasoline and diesel engine exhaust at least with strain TA 100 (Fig. 8). Sample 3 in Figure 8

(gasoline engine) showed exceptionally high mutagenicity of the particulate phase. Also the emission of carbon monoxide (K. E. Egebäck, personal communication) and PAH (U. Stenberg, personal communication) from this experiment indicated abnormal combustion during the test of this vehicle. Under such circumstances the mutagenicity of a gasoline vehicle can be placed in the same group as light-duty diesel vehicles. Another question is if the low particulate emitter giving low mutagenicity of the particulate phase, like LPG and catalyst-equipped vehicles, still will be placed in a low-mutagenicity group when the contribution of gas phase to the total mutagenicity is considered. Such experiments are under way in our laboratory.

Of general importance is, however, the relevance of such a ranking order, as the one outlined above, for the situation *in vivo* and for a further risk evaluation. A crucial point in this connection is the bioavailability of the genotoxic compounds associated with the particulate phase. Several data indicate that this bioavailability may be low (8). If the particulate phase is of little importance as such for genotoxic effects *in vivo*, then the gas phase must be characterized in more detail. The situation may thus turn out to be a question of genotoxic effects of the gas phase. Thus a low particle emitter with a high proportion of genotoxic compounds in the gas phase may constitute a higher risk than a high particle emitter with a low relative part of the genotoxic compounds in the gas phase. Before further conclusions can be drawn on the basis of mutagenicity data *in vitro* a further characterization of the gas phase much be done. Firstly, it must be confirmed that the mutagenicity found in the gas phase is not a result of artifact formation. Secondly, the distribution of components between the particulate phase and the gas phase must be analyzed in more detail, especially in relation to different sampling techniques. Thirdly, more mutagenicity and cancer data, from gas phase exposure *in vivo* to such exhaust emissions that contain the major part of the genotoxic components in the gas phase, are needed.

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